CCPA Reverses Fibrotic Phenotype of Dermal Fibroblasts in Systemic Sclerosis.

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Background: Systemic Sclerosis (SSc) is a connective tissue disease with excessive fibrosis that affects skin and various internal organs. Dermal fibrosis is one of the main manifestation of SSc, however, the therapeutic strategy has not been established merely because the fibrotic mechanisms in the affected skin has not been fully elucidated. Autotaxin (ATX) / lysophosphatidic acid (LPA) axis has emerged as a novel pathogenic factor in various fibrotic disorder including SSc [1]. Cyclic phosphatidic acid (CPA), which is catalyzed by ATX as well as LPA, shows several distinct activities from LPA such as the inhibitory effects of
proliferation, invasion and metastasis of cancer cells [2]. In addition, CPA has been reported to inhibit ATX activity and then, LPA production. These findings suggest that CPA has the potency to ameliorate fibrosis, however, it has not been studied.

**Objectives:** The aim of our study was to investigate the anti-fibrotic property of metabolically stabilized carba derivatives of CPA (CCPA) in Scleroderma dermal fibroblasts.

**Methods:** Primary human dermal fibroblasts obtained from SSc patients or healthy individuals were incubated with CCPA in the presence or absence of TGF-β1 or LPA. The mRNA levels of COL1A1, COL1A2, CTGF, alpha-smooth muscle actin (αSMA), fibronectin (FN), MMP-1, endothelin-1 (ET-1), IL-6 and TGF-β1 were assessed using quantitative real-time RT-PCR. In addition, the protein levels of type I collagen, CTGF and αSMA in cell lysates were evaluated using Western blotting. To examine the effects of CCPA on the Smad and the MAPK signaling pathway, the expression of total and phosphorylated Smad2/3, p38 and ERK1/2 were detected using Western blotting.
**Results:** CCPA significantly decreased the expression of *COL1A1, COL1A2, CTGF, ACTA2* and *FN* mRNA in Scleroderma dermal fibroblasts and healthy dermal fibroblasts stimulated by TGF-β1 in a concentration dependent manner, whereas the expression of *MMP-1* mRNA was significantly increased. Furthermore, the expression of *TGF-β1, ET-1 and IL-6* mRNA was significantly down-regulated at 24h after the treatment with CCPA. The protein levels of type I collagen, CTGF and αSMA were also significantly reduced by CCPA. These effects were shown without the stimulation of LPA. CCPA suppressed the phosphorylation of p38 and ERK1/2, showing that the anti-fibrotic effects of CCPA were partly through MAPK signaling pathway.

**Conclusions:** We demonstrated for the first time that CCPA reversed the fibrotic phenotype of Scleroderma dermal fibroblasts. Intriguingly, CCPA showed anti-fibrotic effects regardless of LPA, suggesting that CCPA has pleiotropic effects other than antagonizing the ATX/LPA axis. CCPA would be a novel therapeutic approach for the treatment of dermal fibrosis of SSc.
References
